

Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world

PETER MILLARD^{1,2,3} and GWEN-AELLE GRELET⁴

¹ Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH, UK

² Landcare Research PO Box 69, Lincoln, New Zealand

³ Corresponding author (p.millard@macaulay.ac.uk)

⁴ Department of Plant and Soil Science, School of Biological Sciences, University of Aberdeen, St Machar Drive, Aberdeen AB24 3UU, UK

Received February 21, 2010; accepted April 13, 2010; published online June 15, 2010

Summary The role of carbon (C) and nitrogen (N) storage by trees will be discussed in terms of uncoupling their growth from resource acquisition. There are profound differences between the physiology of C and N storage. C storage acts as a short-term, temporary buffer when photosynthesis cannot meet current sink demand and remobilization is sink driven. However, the majority of C allocated to non-structural carbohydrates such as starch is not reused so is in fact sequestered, not stored. In contrast, N storage is seasonally programmed, closely linked to tree phenology and operates at temporal scales of months to years, with remobilization being source driven. We examine the ecological significance of N storage and remobilization in terms of regulating plant N use efficiency, allowing trees to uncouple seasonal growth from N uptake by roots and allowing recovery from disturbances such as browsing damage. We also briefly consider the importance of N storage and remobilization in regulating how trees will likely respond to rising atmospheric carbon dioxide concentrations. Most studies of N storage and remobilization have been restricted to small trees growing in a controlled environment where ¹⁵N can be used easily as a tracer for mineral N. We highlight the need to describe and quantify these processes for adult trees in situ where most root N uptake occurs via ectomycorrhizal partners, an approach that now appears feasible for deciduous trees through quantification of the flux of remobilized N in their xylem. This opens new possibilities for studying interactions between N and C allocation in trees and associated mycorrhizal partners, which are likely to be crucial in regulating the response of trees to many aspects of global environmental change.

Keywords: carbon, internal cycling of nitrogen, mycorrhizal fungi, nitrogen uptake, non-structural carbohydrates, phenology, RuBisCo, sequestration.

Introduction

Resource use by trees has often been considered using carbon (C) as a basic currency. Because C₃ photosynthesis is not CO₂ saturated at current atmospheric concentrations and C constitutes about half the dry mass of plants, physiologists have assumed that plant functioning can be considered in terms of the C ‘cost’. The underlying assumption to this approach is that the ability of trees to assimilate and allocate C ultimately regulates their use of other resources and their growth. This C-centric view of tree physiology has tended to dominate our thinking of how trees will respond to aspects of global environmental change, such as rising atmospheric CO₂ levels (as discussed by Körner 2006), temperature (e.g., Adams et al. 2009) or drought (e.g., McDowell et al. 2008). However, this approach assumes that tree growth and functioning are limited by the availability of C. Is this assumption correct? Recent reviews by Körner (2003) and Millard et al. (2007) of a growing body of literature have questioned a C limitation of tree growth under current and future climate conditions. They have used evidence from a range of scales (from leaf biochemistry to ecosystem functioning) to argue that in fact trees are seldom, if ever, C limited. In contrast, it is often reported that tree growth is limited by nutrient availability, particularly nitrogen (N; e.g., Rennenberg et al. 2009) or phosphorous (P), in areas with high N deposition (Akselsson et al. 2008) or ancient forests growing on undisturbed sites (Wardle et al. 2004). Trees accumulate large amounts of C as non-structural carbohydrates (NSC) and lipids (e.g., Hoch et al. 2003, Würth et al. 2005) and the processes regulating how such an abundant resource as C accumulates in trees are quite different from the seasonal allocation of a scarce resource such as N.

Tree growth can use N and C which are derived from several possible sources (Figure 1), broadly categorized as external or internal resources. External N can come from mineralization of

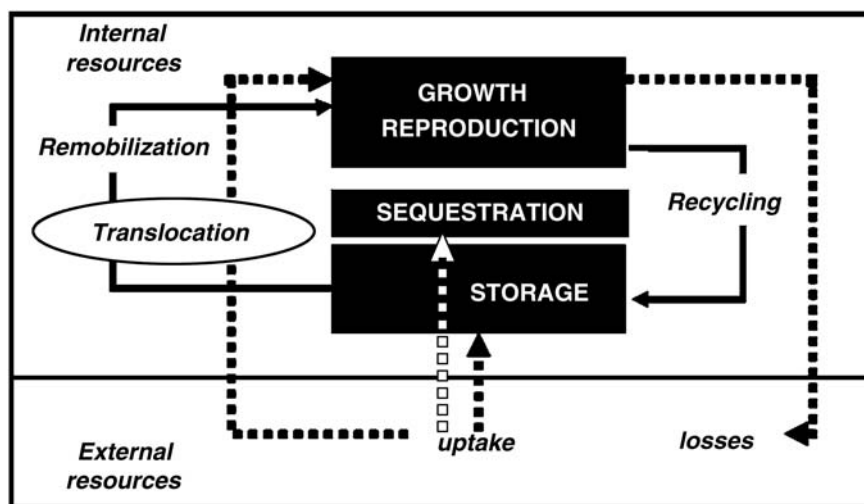


Figure 1. Schematic representation of resource (N and C) acquisition and internal cycling by trees. Exchange of resources between external sources and internal pools in the tree are shown as dotted lines: for uptake directly into storage, sequestration or use in growth, reproduction or other metabolism; for losses via senescence and abscission of leaves and roots. Internal cycling of resources is shown in solid lines, as seasonal remobilization from storage pools via translocation to other tissues for growth, reproduction or other metabolism. Recycling occurs when resources are withdrawn from tissues (usually during senescence, prior to abscission) for storage.

soil organic matter (or fertilizers), microbial fixation of atmospheric N_2 , organic N transferred from mycorrhizal symbionts to tree roots or, in some ecosystems, atmospheric N deposition. External C comes primarily from assimilation of atmospheric CO_2 through photosynthesis. Internal resources are derived from storage through the physiological processes of remobilization and recycling (Figure 1). If a tree is replete with a particular resource, there can also be sequestration, which represents a metabolic dead-end, thereby precluding further use. This review first considers the differences in the physiological processes regulating N and C storage by trees and then discusses the ecological significance of N storage, along with attempts to quantify the contribution N remobilization makes to annual nutrient demand by the tree. First, it is necessary to define exactly what is meant by storage.

Definitions

Storage of resources by plants has been defined in several ways. Millard (1988) defined a concept of N storage by plants, considering N to be stored if it could be remobilized from one tissue for the growth or maintenance of another, while luxury consumption of N leads to accumulation (without further reuse). This definition was extended by Chapin et al. (1990) to consider plant C. They considered that there are three different types of C storage in plants: accumulation, as a consequence of supply exceeding demand for growth, maintenance or reproduction; reserve formation, directly competing with growth or defence, for example, seed production during masting events; and recycling as a consequence of metabolic turnover, for example, protein turnover during leaf senescence (Chapin et al. 1990). Figure 1 builds on these definitions to provide a conceptual model of both

N and C internal cycling in trees. There can be uptake of N or C directly into storage, sequestration or use in growth, reproduction or other metabolism, with losses via senescence and abscission of leaves and roots. The internal cycling of N and C occurs as seasonal remobilization from storage pools via translocation to other tissues for growth, reproduction or other metabolism. Recycling occurs when N or C is withdrawn from tissues (usually during senescence, prior to abscission) for storage. If an accumulated resource is immobilized in plant biomass and cannot be reused, it can then be considered to be sequestered. An example of this is arginine accumulation in the needles of coniferous trees in response to atmospheric N deposition. In this case, arginine cannot be reused or withdrawn from the needles (Näsholm 1994), so the accumulation represents sequestration of N. Thus, it is necessary to demonstrate subsequent remobilization and reuse of N in order to be able to assess storage. These definitions are important because they emphasize the dynamic nature of storage. Build-up of NSC pools in trees represents accumulation (but not necessarily storage) of C and is dependent upon the rate of current assimilation. For N, the ability for storage is not dependent upon the external supply, which only influences the amount of N stored. Instead, N storage and remobilization are closely linked to phenological events in annual growth cycles, with N requirements for growth, maintenance and defence being met by either (i) remobilization of N from storage or (ii) direct root uptake of external N. The balance between the two determines both the rate of N losses and the N use efficiency (NUE) of a tree and thereby contributes to its fitness in N-poor environments. Therefore, there are profound differences in the physiology of C and N use by trees (Table 1), which question whether the same strategies of storage and remobilization apply to both.

Table 1. Physiological characteristics of N and C storage and remobilization by trees.

N storage and remobilization	C storage and remobilization
Stores can fill while canopy senescens or is dormant	Storage determined by photosynthetic rates
Storage seasonally programmed, linked with phenology	Sequestration to avoid down regulation of photosynthesis
Storage occurs in specific tissues/organs	Accumulation of C occurs throughout the tree
Stores empty fully during periods of remobilization	'Stores' never empty fully
Remobilization source driven	Remobilization is sink driven
Remobilization unaffected by current N supply	Remobilization affected by current photosynthesis

Differences in the physiology of C and N storage

Sites and turnover of C and N storage pools

C accumulates in trees as NSC pools during periods of canopy photosynthesis, which can be depleted during periods of rapid growth or when respiration exceeds photosynthesis. In deciduous trees, this means that NSC levels increase during summer (Sauter and van Cleve 1994) and are often at a maximum in late summer/autumn and a minimum in late spring (e.g., Hoch et al. 2003, Spann et al. 2008). In evergreen trees, NSC pools are replete in spring before bud break and lowest in late summer (Hoch et al. 2003). The NSC accumulate throughout the plant (e.g., Hoch et al. 2003, Würth et al. 2005, Palacio et al. 2008, Spann et al. 2008), including in leaves, stems, trunk wood and coarse and fine roots.

N is stored in trees in a wide range of different vegetative storage proteins, such as bark storage proteins (BSP) (Cooke and Weih 2005). In addition, a range of metabolically active proteins, such as RuBisCo, can serve as N storage pools (Millard et al. 2007). The sites of N storage by trees are, however, restricted to specific organs within an individual species, dependent upon leaf habit (Table 2). Deciduous species tend to store N in the wood and bark of roots or the trunk, while evergreen species store N in the youngest age class of foliage, although there are some exceptions to this (e.g., *Northofagus fusca* storing N in the roots; Table 2). In contrast to NSC pools, N is never stored throughout the whole tree and, during periods of N remobilization, all of the N storage pools turn over. This is seen, for example, by the disappearance of BSP from the branches (Tian et al. 2003), trunk (Cooke and Weih 2005) or roots (Langheinrich and Tischner 1991) of deciduous species by the summer or the selective loss of RuBisCo from senescing leaves (Millard and Thomson 1989). In coniferous evergreen trees, N is stored mainly in the youngest age class of needle (e.g., Nambiar and Fife 1987, Millard and Proe 1992, Millard et al. 2001). Much of this N is stored in photosynthetic proteins, especially RuBisCo as shown by a specific decrease in needle RuBisCo content during spring flushing (Camm 1993), while in autumn, an amount of RuBisCo far in excess of that required for photosynthesis can be synthesized (Millard et al. 2007). This implies that there are two pools of RuBisCo in the needles, perhaps separated by their activation state (Manter et al. 2005), with the inactive pool turning over during spring remobilization of N.

N allocation to storage is programmed seasonally and is, therefore, intimately linked to tree phenology. The allocation of N to storage pools such as BSP is triggered by specific environmental cues, notably short days (Coleman et al. 1991) and also low temperature (van Cleve and Apel 1993). For temperate trees, these are conditions experienced in autumn, when N uptake by roots can be rapid and contribute directly to storage, despite canopy senescence (Millard and Thomson 1989, Millard and Proe 1991), possibly explaining why BSP accumulation can also be triggered experimentally at other times of the year by providing high N levels to the plant (van Cleve and Apel 1993, Zhu and Coleman 2001). In contrast to seasonally programmed N storage, C allocation to NSC is a passive buffer and patterns of NSC turnover are relatively small and not, or only weakly, associated with reproductive phenology (Körner 2003).

Source- versus sink-driven remobilization

Following on from these differences in N and C accumulation in trees, another difference between NSC and N storage pools is that remobilization of C is sink driven, while that of N is source driven. These differences are manifested by the extent of any seasonal remobilization of C from NSC being dependent on the sink strength imposed by new growth. Several lines of evidence support this. Seasonal fluctuations in the levels of NSC have been found in many species, especially in seedlings (e.g., Gansert and Sprick 1998). C remobilization in deciduous trees has been demonstrated as an important process during bud burst (Maurel et al. 2004). There is a significant turnover of NSC pools from trunk wood (e.g., Spann et al. 2008) and branch sapwood (e.g., Spann et al. 2008, Schädel et al. 2009) just before bud burst in deciduous, temperate trees, which can provide the majority of C used for early spring growth (Vizoso et al. 2008). In boreal species with a short growing season, up to about 40% of the C used for new leaf growth can be provided by remobilization (Kagawa et al. 2006a), which also contributes to early wood formation (Kagawa et al. 2006b). C remobilization can also be important for recovery from winter embolism (Ameglio et al. 2004), fire (Schutz et al. 2009) and herbivory over the timescale of a few months (Palacio et al. 2008). Some tree species from Mediterranean ecosystems also remobilize C from NSC during periods of summer drought, although others do not (e.g., Sanz-Pérez et al. 2009). Experiments that have manipulated sink strength through N supply (Vizoso et al. 2008) or

Table 2. An analysis of studies that have quantified the contribution of N remobilization to the seasonal growth of trees. Studies have been included when N used for leaf growth was assessed over a whole growing season or at least until the first flush of leaf growth had finished.

Leaf habit	Species	Tree age (years)	Main site of N storage	Proportion of N for new shoot growth remobilized from storage	Method	Reference
Deciduous broadleaf tree	<i>Acer pseudoplatanus</i>	4	Roots and	37–48% (\pm N)	$^{15}\text{N}^a$	Millard and Proe 1991
		9	stem	\approx 33%	$^{15}\text{N}^b$	Millard 1994
	<i>Betula pendula</i>	4	Woody	48%	$^{15}\text{N}^a$	Millard et al. 2001
		4	roots/stem	37%	$^{15}\text{N}^a$	Millard et al. 1998
		4		50–54% (\pm N)	$^{15}\text{N}^a$	Wendler and Millard 1996
		4	ND	52–72% (competition with herbaceous spp.)	$^{15}\text{N}^b$	Millett et al. 2005
	<i>Fraxinus excelsior</i>	4	ND	17–29%	$^{15}\text{N}^a$	Millard 1993
	<i>Fagus sylvatica</i>	4	Stem and coarse roots	18%	$^{15}\text{N}^a$	Dyckmans and Flessa 2001
	<i>Jugulans nigra</i> \times <i>regia</i>	3	Stem and coarse roots	54%	$^{15}\text{N}^a$	Frak et al. 2002
	<i>Jugulans regia</i>	9	roots	60%	^{14}N /budget	Weinbaum and Van Kessel 1998
	<i>Malus domestica</i>	Mature		41–72% (time)	^{14}N /budget	Deng et al. 1989
		4	ND	61–87% (\pm N)	$^{15}\text{N}^b$	Guak et al. 2003
		5	ND	87% (spur leaves)	$^{15}\text{N}^b$	Neilsen et al. 1997
		3	Stem	52% (shoot leaves)		
				28–34%	$^{15}\text{N}^b$	Millard and Thomson 1989
		2	Stem	18–54% (\pm N)	$^{15}\text{N}^b$	Millard and Neilsen 1989
	<i>Populus</i> spp.	Various	BSP	ND	—	Cooke and Weih 2005
	<i>Prunus avium</i>	2	ND	14–26% (\pm N previous year)	$^{15}\text{N}^b$	Grassi et al. 2002
	<i>Prunus persica</i>	2	ND	72–80% (different varieties)	$^{15}\text{N}^b$	Policarpo et al. 2002
	<i>Pyrus communis</i>	3	ND	47%	$^{15}\text{N}^b$	Quartieri et al. 2002
	<i>Quercus robur</i>	1	Stems/large roots	80–100% (\pm N)	$^{15}\text{N}^a$	Vizoso et al. 2008
	<i>Quercus pyrenaica</i>	2–4	Woody tissues	7–82% (different years)	Budget	Silla and Escudero 2003
Marcescent/evergreen tree	<i>Sorbus aucuparia</i>	4	Woody roots/stem	32%	$^{15}\text{N}^a$	Millard et al. 2001
	<i>Nothofagus fusca</i>	5	Roots	40%	$^{15}\text{N}^a$	Stephens et al. 2001
	<i>Quercus faginea</i>	2–4	Woody tissues/previous-year leaves	50–88% (different years)	Budget	Silla and Escudero 2003
Evergreen, broadleaf tree	<i>Eucalyptus globulus</i>	2	Older leaves	10–44% (\pm N)	Budget	Wendler et al. 1995
	<i>Quercus ilex</i>	2–4	Woody tissues/previous-year leaves	17–64% (different years)	Budget	Silla and Escudero 2003
Evergreen, coniferous tree	<i>Picea mariana</i>	1	Old shoots	13–100% (level of N over 2 years)	$^{15}\text{N}^b$	Salifu and Timmer 2003
		1	Old needles/stem	3–59% (previous N supply)	Budget	Mailk and Timmer 1997
	<i>Picea sitchensis</i>	4	Previous-year needles	9–46% (\pm N)	$^{15}\text{N}^b$	Millard and Proe 1992
		5	Previous-year needles	24–39% (\pm N)	$^{15}\text{N}^b$	Millard and Proe 1993
	<i>Pinus radiata</i>	4	Previous-year needles	57–31% (\pm N)	Budget	Nambiar and Fife 1987
	<i>Pinus sylvestris</i>	4	ND	44–83% (\pm N)	$^{15}\text{N}^b$	Proe et al. 2000
		4	Previous-year needles	25–37% (\pm defoliation)	$^{15}\text{N}^a$	Millard et al. 2001
	<i>Prumnopitys ferruginea</i>	4	Foliage	36–93% (\pm N)	$^{15}\text{N}^b$	Carswell et al. 2003

Continued

Table 2. Continued

Leaf habit	Species	Tree age (years)	Main site of N storage	Proportion of N for new shoot growth remobilized from storage	Method	Reference
Deciduous shrub	<i>Vaccinium ashei</i>	ND	Roots and shoots	≈50% (fruits) ≈20% (vegetative growth)	Budget	Birkhold and Darnell 1993
	<i>Vaccinium myrtillus</i>	>2	Roots/woody stems	15–48% (±N)	¹⁵ N ^b	Grelet et al. 2001
Semi-deciduous shrub	<i>Buddleia davidii</i>	3	Old leaves	50–60% (±defoliation)	¹⁵ N ^b	Thomas et al. 2008
Evergreen shrub	<i>Rhododendron ferrugineum</i>	5	Current-year leaves	ND	¹⁵ N and budget	Pornon and Lamaze 2007
		ND	Leaves/wood	60–72% (low or high soil fertility)	Budget	Marty et al. 2009
	<i>Vaccinium vitis-idaea</i>	>2	Old leaves/green stems	9–23% (±N)	¹⁵ N ^b	Grelet et al. 2001

^a¹⁵N applied the year before sampling, so only remobilization of N taken up the previous year quantified.

^b¹⁵N applied the year of sampling, so remobilization of all N quantified.

ND, not determined.

defoliation and bud removal (e.g., Li et al. 2002) have shown that C remobilization from NSC is determined by the balance between current photosynthesis and sink strength for new growth. However, in each of the examples above, remobilization of C from NSC pools has been a temporary source of C because the rate of current assimilation was not sufficient to meet the sink demands for growth, with current assimilation the primary source of C used for growth. Therefore, while C can be remobilized from storage to support growth, this can be considered a consequence of a source–sink imbalance, rather than a seasonally programmed process.

In contrast, N remobilization is seasonally programmed and depends upon the amount of N in store, not the amount of new growth or current rate of N uptake. This has been demonstrated in several studies for a range of species. First, bud removal to simulate winter browsing of species such as *Betula pubescens* leads subsequently to compensatory leaf growth, which Lehtilä et al. (2000) suggested was explained by the redistribution of a predetermined amount of resource to existing buds. This has been confirmed in studies that have used ¹⁵N to quantify N remobilization and found that, if sink strength for growth is altered by removing dormant buds, then the amount of N subsequently remobilized per remaining bud is increased (Millard et al. 2001, Millett et al. 2005). The second line of evidence comes from studies where N storage pools have been manipulated by contrasting N supplies between years. The amount of N remobilized in the second year was dependent upon N supply in the first year, but unaffected by N availability during the second in both deciduous (Millard and Neilsen 1989, Millard and Proe 1991, Millard 1993, Cheng and Fuchigami 2002) and coniferous evergreen species (Millard and Proe 1993), despite the second-year N supply affecting growth. Thus, N remobilization is source driven by the size of the storage pool, not the sink strength for growth.

Most C is sequestered, N is stored

There is now plenty of evidence to suggest that much of the NSC accumulating in trees represents sequestration, not storage. Trees accumulate large pools of NSC, enough to replace the entire canopy four times in temperate species (Hoch et al. 2003) or represent 8% of total forest biomass in a tropical forest (Würth et al. 2005). Despite some seasonal variations in NSC levels, the pools are never fully depleted (Hoch et al. 2003, Würth et al. 2005, Spann et al. 2008). Indeed, in his synthesis of a 10-year study of NSC pools in trees, Körner (2003) reported that there was not a single case where pools came even close to depletion during periods of high sink demand. Even when tree growth is constrained to a short season by temperature (e.g., at the tree line), accumulation of NSC suggests that C availability is not a limitation to growth (Hoch and Körner 2003). Tree NSC pools have also been shown to be unaffected by long-term browsing damage (Palacio et al. 2008). In addition, if the supply of photosynthate is cut, pools of NSC remain that cannot be utilized, as seen in roots, for example, following phloem girdling (Bhupinderpal-Singh et al. 2003), or branches, following severe water stress (Bréda et al. 2006) or girdling and defoliation (Hoch 2005). These studies of NSC pools suggest that, while trees store N (as shown by the disappearance of N storage pools after remobilization during flushing), the majority of C accumulating in their tissues as NSC is not reusable by the tree, so is in fact sequestered. This has been confirmed by studies that have considered both C and N remobilization and found spring growth determined mainly by reserve N, not carbohydrates (Cheng and Fuchigami 2002).

The study of NSC accumulation in trees in relation to their growth and C allocation has become quite popular (e.g., Hoch and Körner 2003, Hoch et al. 2003, Würth et al. 2005, Bréda et al. 2006, Palacio et al. 2008, Spann et al.

2008, Schädel et al. 2009). However, it is clear that measuring NSC pools cannot be used to quantify the C storage capacity of a tree because a sizeable but unknown proportion appears to be sequestered, not stored C. Given that tree growth is not C limited (Körner 2003, Millard et al. 2007, Palacio et al. 2008), it is questionable how useful it is to understand the physiology of C allocation to NSC. In contrast, N storage and remobilization are important processes regulating the growth potential of a tree. We next consider the ecological significance of N storage and remobilization by trees.

Ecological significance of nitrogen storage and remobilization

Nitrogen use efficiency

The ability to store and remobilize N are considered key processes for the NUE of plants. Originally, Vitousek (1982) defined NUE as the 'amount of organic matter lost or permanently stored in the plant per unit of nutrient lost or permanently stored in the plant' as measured through above-ground litter returns. According to this definition, a partial index of NUE is the inverse of N concentration in the leaf litter. This allowed the comparison of NUE for several forest stands, relating them to the total amount of N lost in litterfall (Vitousek 1982) or mean annual rates of soil N mineralization (Birk and Vitousek 1986). These studies are based on the assumptions that N cycling reflects N availability for plant uptake and/or mineral N is the main source of soil N for tree growth. However, the assumption that mineral N represents the main source of soil N is now challenged, as the ability to access organic N has been shown for many species of plants, including trees (reviewed by Näsholm et al. 2008). Ectomycorrhizal fungi have a capacity for mobilizing N and P from soil organic matter, which could satisfy a considerable proportion of the external N requirement of the tree (Read and Perez-Moreno 2003), while potentially bypassing mineralization processes by free-living microbes (Talbot et al. 2008). In addition, Vitousek's approach does not account for below-ground losses of C and N, which might be substantial for both C, through rhizodeposition and transfer to symbiotic mycorrhizal fungi, and N, through root senescence because N resorption from senescing roots is generally negligible (e.g., Nambiar 1987, Aerts 1990).

Berendse and Aerts (1987) redefined NUE as the product of N productivity (A_N , dry matter production per unit of N in the plant) and the mean residence time of N (MRT_N) in the plant. Berendse and Aerts (1987) also suggested that A_N and MRT_N would be inversely correlated because of trade-offs between traits leading to fast growth rates and those reducing N losses, restricting the variation in NUE between species. As a consequence, nutrient-rich habitats would be dominated by faster-growing species (with high A_N) with rapid nutrient turnover (short MRT_N), such as deciduous species. In contrast, in less-fertile systems, slower-growing species (with

low A_N), which retained their nutrients (long MRT_N), such as evergreen species, would predominate. Such a tradeoff between A_N and MRT_N has been measured in some woodland ecosystems (e.g., Silla and Escudero 2004). Of course, one advantage of conserving N within the plant in N-limited environments is to reduce competition with other individuals for external N acquisition and ultimately reduce availability of N to other species.

Following the work of Vitousek (1982) and Berendse and Aerts (1987), NUE emerged as a core concept for the analysis of the relationship between C gain and the flux of N through plants and was used to try to explain the predominance of evergreen species in nutrient-poor habitats (e.g., Aerts 1995). While it is a useful concept, NUE has C as the basic currency. This may have limited ecological relevance to forest ecosystems that are not C limited. Patterns of N storage and remobilization depend to a great extent upon leaf habit, with differences in sites of N storage between deciduous broadleaf, evergreen broadleaf and coniferous evergreen species for example (Table 2). It is probably more important to understand these differences than to measure NUE in order to understand tree fitness for a particular environment. For example, an analysis of NUE and its underlying components suggests that NUE is maximized by synthesizing low-N leaves, most apparent in evergreen trees confined to nutrient-poor habitats (Aerts 1995), which has in turn been interpreted as a limitation on plant growth rate due to low C assimilation rates (Aerts and Chapin 2000). However, given the abundance of NSC in trees discussed above, it is more likely that N supply, rather than C assimilation rates, will limit their growth. Indeed, some evergreen species even have plasticity in leaf life span and pattern of N remobilization, shortening leaf life span in response to low soil fertility (Marty et al. 2009). This is counter to what would be predicted by an analysis of their NUE.

Uncoupling growth from N uptake

Tree growth in the spring often starts before N uptake from the soil by roots. As a result, initial leaf growth following bud burst utilizes remobilized N. The extent to which this occurs is demonstrated in Table 2, which shows the proportion of N for new shoot growth remobilized from storage for a range of tree species having different leaf habits.

Experiments utilizing ^{15}N to quantify spring uptake of N by roots have shown that, for a range of species, N remobilization for above-ground growth in spring occurs before utilization of N taken up by roots. These include deciduous species, such as *Malus domestica* (Nielsen et al. 1997, Guak et al. 2003), *Populus trichocarpa* (Millard et al. 2006), *Prunus avium* (Grassi et al. 2002), *Pyrus communis* (Tagliavini et al. 1997) and *Sorbus aucuparia* (Millard et al. 2001); marcescent/evergreen species, such as *Nothofagus fusca* (Stephens et al. 2001); and coniferous evergreens, such as *Picea sitchensis* (Millard and Proe 1993). In these species, N remobilization can occur for typically 20–30 days before the roots become active for N uptake. In a few species (e.g., *S. aucuparia*), remobilization

has completely finished before any root uptake of N occurs, even if trees are supplied with an adequate supply of mineral N in the soil. In contrast, other species have been shown to commence N uptake by their roots concomitantly with N remobilization. These include deciduous *Juglans nigra* × *regia* (Frak et al. 2002), *Betula pendula* (Millard et al. 2001) and evergreen *Pinus sylvestris* (Millard et al. 2001). The majority of studies have used sapling trees growing in sand culture in pots and so representing small plants, with constrained roots of an uncertain mycorrhizal status and often unrealistic similarities between soil and air temperature. However, there are a few studies that have applied ^{15}N -labelled mineral fertilizer to larger, undisturbed trees growing in the field, such as *Acer pseudoplatanus* and *P. sitchensis* (Millard 1994) and *P. avium* (Millard et al. 2006). While these studies were not quantitative because ^{15}N -labelled fertilizers were diluted with the native soil N, meaning that total N uptake by roots could not be measured, they do confirm the pattern of N remobilization occurring before rapid root uptake of N.

One reason for remobilization occurring before root uptake of N might be that air temperature rises faster in the spring than the soil warms. As a consequence, bud burst and flushing occurs while the soil has slow rates of N mineralization and so a low availability of mineral N. In this case, remobilization would be a mechanism for supplying N when root N uptake is limited. This would be important in relatively fertile systems where mineral N is the predominant form taken up by roots. However, as discussed above, in less-fertile boreal and temperate ecosystems, a considerable proportion of the N demand of the trees could be met by ectomycorrhizal fungi mobilizing N and from soil organic matter (Read and Perez-Moreno 2003). In these systems, is N storage and remobilization a mechanism to uncouple growth from being reliant on the activity of mycorrhizal symbionts?

The extent to which N uptake by tree roots via ectomycorrhizal fungi occurs in spring, concomitant with N remobilization, is unknown. However, during spring and early summer, C allocation below ground is slower than later on in the summer/autumn. Evidence for this comes from several sources. First, $^{13}\text{CO}_2$ pulse chase studies in a boreal forest have shown that C assimilated just after bud burst was allocated above-ground, with only 2–4% allocated to roots and their symbionts (Kagawa et al. 2006a). In contrast, 1 month later, some 32–44% of current assimilates were allocated to roots. The second line of evidence comes from experiments in a 120-year-old *Picea abies* forest that used tree girdling to partition autotrophic soil respiration into autotrophic (R_A) and heterotrophic (R_H) components. While R_H was relatively constant during the growing season, R_A was initially a very small proportion of soil respiration (Högberg et al. 2009), suggesting that, at the start of the growing season, there was little below-ground allocation of C. There could be several reasons for little C allocation below ground in the spring. Cool soil temperatures might limit root growth and mycorrhizal

activity, thereby reducing the C sink strength. Certainly, soil respiration is driven by temperature, but in their boreal system, Högberg et al. (2009) found a hysteresis in the relationship such that season, not just temperature, was an important driver. A mycogenic explanation for the lack of C allocation to roots in spring could be that the mycorrhizal fungi themselves are nutrient limited in spring and, therefore, are not engaging in reciprocal C/N transfer with their host tree. If this was the case, less C allocation below ground would stop the fungi from becoming ‘cheaters’, with N remobilization used as a transient alternative to fungal-derived N. The alternative phytocentric view would be that, in some species, bud burst provides such a strong C sink in the plant that, despite large NSC pools, there is insufficient C available for below-ground allocation and transfer to the fungi, so remobilization is a necessary temporary source of N until current assimilation builds up sufficient C to allow root activity and their N supply to commence. However, this latter scenario is unlikely because it would suggest that evergreen species (which retain a C assimilatory capacity throughout the year) would be less likely to rely upon N remobilization in spring than deciduous species. However, there is no evidence for this (Table 2).

Recovery from disturbance

There is a wide range of disturbances that trees can experience. These include herbivory by animals, ranging in size from small insects to large mammals; occasional fire in many forest systems that are naturally fire regenerated; changeable weather patterns, including seasonal droughts in, for example, Mediterranean systems; and global environmental change, including rising atmospheric CO_2 levels and N deposition. The use a tree can make of stored N to support growth following a disturbance will depend on how vulnerable the store was to the disturbance itself. For example, the ability to store N in roots is important for some trees responding to either drought stress (Silla and Escudero 2006) or browsing damage by large herbivores (Millard et al. 2001) because N stored in leaves is vulnerable to loss by leaf shedding or defoliation. Seasonal storage and remobilization of N also act as buffers against short-term fluctuations in N supply, caused, for example, by changeable weather patterns from year to year. In order to illustrate the ecophysiological importance of N storage and remobilization in recovering from a disturbance, we will briefly consider two specific examples: recovery from herbivory and how N storage by trees will likely respond to increasing atmospheric CO_2 levels.

Herbivory Browsing is an important factor inhibiting woodland regeneration in many parts of the world (Gill 2006). One consequence of browsing during spring or summer is a decrease in leaf area resulting in a concomitant loss in C assimilation. Therefore, many studies have considered the consequences of herbivory on the C physiology of trees (e.g., Hoogesteger and Karlsson 1992, Honkanen et al. 1999) and it has been suggested

several times that C limitation is the cause of reduced growth following browsing (e.g., [Hoogesteger and Karlsson 1992](#), [Crête and Doucet 1998](#)). However, other studies have suggested that trees subjected to long-term browsing were not C limited ([Palacio et al. 2008](#)) and that trees could even up-regulate C assimilation to compensate for losses due to herbivory (e.g., [Dungan et al. 2007](#)).

The ability of different tree species for compensatory growth (and C assimilation) following defoliation depends upon several aspects of their physiology. The potential for compensatory growth depends on bud phenology and whether a tree has a fixed or indeterminate growth pattern. As a consequence, species with an indeterminate growth pattern and with buds capable of neoformed growth will have a greater potential capacity for compensatory growth than those with a fixed growth pattern ([Millard et al. 2001](#)), particularly if herbivory occurs during the spring or summer after flushing. However, the ability to remobilize N from storage is an important factor in allowing the tree to realize the growth potential determined by their bud phenology.

The C/nutrient balance hypothesis ([Bryant et al. 1983](#)) suggests that species growing on fertile sites will respond to herbivory by utilizing stored resources for compensatory growth, whereas slower-growing species or those adapted to less-fertile sites will instead protect their leaves by investing a greater proportion of their C in anti-herbivory defences. This is a somewhat C-centric view of how trees respond to herbivory. When tree growth is limited by the availability of N, they rely on storage and remobilization of N to meet their requirements for annual growth to a greater extent than when well supplied with nutrients ([Table 2](#)). Long-lived, slow-growing evergreen species are commonly found in the most nutrient-poor sites and, because they store N in their foliage ([Table 2](#)), their reserves are susceptible to loss by herbivory. In addition to protecting leaves per se, it is likely that anti-herbivory compounds serve primarily to protect the nutrients stored within them. In other words, protecting N resources is likely to be more important than maintaining a capacity for C assimilation.

Rising atmospheric CO₂ Another form of disturbance trees have to face is rapidly rising atmospheric CO₂ levels, which is having the effect of priming ecosystems with extra C. There is a large literature dealing with the response of trees and forests to elevated CO₂. A key finding is that, when elevated CO₂ is provided experimentally, increases in NPP are found, although initial responses in above-ground growth decline through time (e.g., [Körner 2006](#)). A progressive N limitation to growth under elevated CO₂ has been suggested as C and N are sequestered in woody biomass and soil organic matter ([Johnson 2006](#)). Therefore, the ability to internally cycle N might be an important mechanism for trees to cope with rising CO₂ levels.

N remobilization by deciduous trees appears to be unaffected by elevated CO₂ ([Dyckmans and Flessa 2005](#), [Vizoso et al. 2008](#)). However, coniferous evergreen trees, which

store N in RuBisCo, might be particularly susceptible to elevated CO₂. In N-limited ecosystems, the key effect of the plants' response to elevated CO₂ is an increase in efficiency of N use for photosynthesis due to the increased carboxylation efficiency of RuBisCo ([Drake et al. 1997](#)). This in turn leads to a selective loss of RuBisCo enzyme under elevated CO₂, increasing NUE ([Long et al. 2004](#)), without necessarily a concomitant change in leaf C assimilation rate. In the medium term, if less investment in RuBisCo protein compromises the ability to store and internally cycle N (the limiting resource for growth), this would contribute to the 'progressive N limitation' under elevated CO₂ (e.g., [Johnson 2006](#)). However, it is interesting that the acclimation of RuBisCo to elevated CO₂ in 1-year-old needles of pines was not found in the current-year needles ([Tissue et al. 2001](#), [Rogers and Ellsworth 2002](#)). As current needles are the main site of N storage in pines ([Millard et al. 2001](#)), this suggests that acclimation does not occur until after RuBisCo turnover to release N from storage from the youngest needles at the onset of spring growth. Acclimation would, therefore, not occur at the expense of N storage by the tree. [Temperton et al. \(2003\)](#) confirmed this by showing that elevated CO₂ had no effect on N remobilization by young *P. sylvestris* seedlings, although the consequences for larger trees are unknown.

From the above discussion, it is clear that N storage and remobilization are of considerable ecological and physiological significance to trees. We now turn to consider the quantification of N remobilization and its contribution to supply the N demand for growth each year.

Contribution of N storage and remobilization to annual growth

[Table 2](#) shows the proportion of N for new shoot growth that was remobilized from storage (PN_{REM}) for a range of tree species having different leaf habits. The values reported come predominantly from experiments that have used ¹⁵N to quantify N remobilization, the majority studying saplings growing in sand culture. Two different methods for quantifying N remobilization using ¹⁵N have been utilized in these studies. The isotopes were either applied the year before sampling (so only quantifying remobilization of N taken up the previous year) or supplied to trees the same year as they were sampled (thereby allowing remobilization of N from all previous years to be quantified). A number of budget studies have also been included in [Table 2](#) where the total N remobilization to annual growth was estimated.

The range of values reported for PN_{REM} is very variable ([Table 2](#)). This variability is due to many factors, including the age of the trees, the tree N status and the method used to quantify remobilization. Overall, there were no evident differences in PN_{REM} between evergreen and deciduous species. Some species showed an almost complete dependence on N remobilization for their growth (e.g., deciduous *Quercus robur*; [Table 2](#)), while others showed a wide range

Table 3. Translocation of amino compounds in the xylem sap of trees as a consequence of N remobilization in the spring. In each case, the amino compound has been identified by using ^{15}N labelling, with the principal compound rendered in bold text.

Species	Amino acids	Reference
<i>Acer pseudoplatanus</i>	Asn , Gln	Millard, unpublished
<i>Betula pendula</i>	Cit , Gln	Millard et al. 1998
<i>Jugulans nigra</i> \times <i>regia</i>	Arg , Cit, Glu	Frak et al. 2002
<i>Malus domestica</i>	Asn , Gln, Asp	Malaguti et al. 2001
	Asn , Asp, Gln	Guak et al. 2003
<i>Nothofagus fusca</i>	Gln	Stephens et al., unpublished
<i>Populus trichocarpa</i> \times <i>balsamifera</i>	Gln	Millard et al. 2006
<i>Prunus avium</i>	Gln , Asn, Asp	Grassi et al. 2002
	Gln , Asn	Millard et al. 2006

of PN_{REM} (e.g., deciduous *Quercus pyrenaica* and evergreen *Picea marianan* or *Prumnopitys ferruginea*; Table 2). In the majority of studies, a variation in PN_{REM} for an individual species was measured after a manipulation of the N supply, with PN_{REM} being greater under low N than for well-fertilized plants, emphasizing the importance of N storage for tree growth in N-limited systems. However, other factors also caused variations in PN_{REM} , including variable weather between years, defoliation and competition with herbaceous species. Given that the oldest trees studied in Table 2 were only 9 years old, the synthesis shows that N remobilization is quantitatively important for the growth of young trees and is a process which is greatly influenced by a wide range of environmental parameters.

It is well established that remobilization becomes quantitatively more significant as trees grow (Miller and Miller 1987) because, as they develop, the rate of N uptake slows, while their potential storage capacity for N increases (Miller 1986). Quantification of N remobilization from isotopic studies with small trees will not, therefore, necessarily scale to larger trees growing in soil. In addition, all of the studies reported in Table 2 have only considered uptake of mineral N by tree roots while, as discussed above, in many systems, uptake of organic N might predominate. There is, therefore, a pressing need to develop robust and accurate methods for measuring N remobilization in larger trees growing in soil with a full mycorrhizal community on their roots.

Measuring PN_{REM} in a mature tree is difficult. Two basic approaches have been used to date: first, constructing tree N budgets from sequential sampling or, second, using ^{15}N to quantify N uptake within a season and calculating PN_{REM} by difference. N budgets constructed for larger trees growing in soil are often imprecise. For example, several have attempted to quantify remobilization by measuring N withdrawal from senescing leaves (e.g., Côté and Camiré 1987, Helmisaari 1992), without accounting for N uptake in the autumn contributing directly to storage, which has been shown to occur in a range of species (e.g., Millard and Thomson 1989, Millard and Proe 1991). Other studies have calculated the net loss from older tissues (e.g., Nambiar and Fife 1987, Lal et al. 2001), although the timing of sampling can be critical to be able to measure the outcome of physiological processes such as remobilization, which can occur over a

relatively short time period. Because N budget studies only measure the result of net fluxes, they can also underestimate PN_{REM} (Proe et al. 2000). Even if ^{15}N labelling is used, budget studies in the field are difficult to interpret because of the impossibility of uniformly labelling all the soil N pools (including organic N pools) and the incomplete recovery of tree roots (estimated as being <65% of root mass; e.g., Le Goff and Ottorini 2001).

An alternative approach to measuring N remobilization by trees has been to consider the flux of N in the xylem during bud burst and leaf growth. In evergreen trees, N is often stored in the leaves during winter (Table 2). During spring remobilization, N is translocated from old to new leaves. However, this translocation is likely to be restricted to short pathways, the N demand of newly grown leaves being met by translocation from old leaves nearby. In contrast, deciduous trees store N during the winter in their roots and trunk (Table 2). Hence, in the spring, remobilized N is offloaded in the xylem and can be translocated over the entire height of the tree.

The predominant amino compounds translocated in the xylem sap of trees are the amides glutamine and asparagine, with arginine, citrulline and glutamic acid also found in some species (reviewed by Pfautsch et al. 2009). Several studies of temperate trees have measured a sharp peak in the concentration of N in the xylem sap during bud burst, which was attributed to N remobilization (e.g., Glavac and Jochheim 1993, Schneider et al. 1994). In tropical forests, seasonal differences between amides or arginine as the main N compound in xylem sap was interpreted as a switch between root uptake of N in the wet season and N remobilization in the dry season (Schmidt and Stewart 1998). Use of ^{15}N tracers to label N storage pools in young, temperate trees has confirmed that remobilization in the spring coincides with peaks in xylem sap amino acid concentrations. Specific amino acids and amides translocated due to remobilization have been identified by their ^{15}N -labelling pattern, as summarized in Table 3. Subsequently, the flux of remobilized N in the xylem has been calculated by measuring sap flux and the concentration of amino acids translocated during remobilization. This new approach to quantifying remobilization gave good agreement with quantification using ^{15}N and destructive harvesting for young *P. avium* and *Jugulans nigra* \times *regia*

grown in sand culture (Grassi et al. 2002, Frak et al. 2002) and young *M. domestica* grown in soil (Guak et al. 2003). The potential for the technique was also demonstrated in larger, mature trees by Millard et al. (2006) who demonstrated using ^{15}N that similar patterns of glutamine translocation as a consequence of spring remobilization of N occurred in *P. avium* and *P. trichocarpa* \times *balsamifera*. Subsequently, Pfautsch et al. (2009) successfully used this approach to quantify the annual N flux in the xylem of *Eucalyptus regnans*. This opens the intriguing possibility of being able to quantify N remobilization by large, deciduous trees growing in the field accurately for the first time, without the need for destructive harvesting or the use of ^{15}N tracers.

Future research

Much of the literature on tree physiology is still based on a C-centric view of the world. We have questioned the relevance of such a view to understanding the functioning of trees, based on the fact that tree growth is seldom, if ever, limited by the availability of C. In order to be able to assess the C status of a tree, further research is certainly needed to be able to measure the proportion of NSC pools in a tree that are stored as opposed to sequestered. However, as trees are replete with C, their C status is not the most important aspect of their physiology to understand. In contrast, there is a growing body of evidence for the importance of N storage and remobilization for tree growth, while our knowledge of P dynamics in trees is still only rudimentary. As the majority of the quantitative studies of N storage and remobilization have, by necessity, used young trees growing in artificial conditions, there is now a need to describe and quantify these processes for adult trees in situ where most root N uptake might occur via ectomycorrhizal partners. Such an approach now appears potentially feasible for deciduous trees through quantification of the flux of remobilized N in their xylem. This opens new possibilities for studying interactions between N and C allocation in trees and associated mycorrhizal partners, which are likely to be crucial in regulating the response of trees to many aspects of global environmental change. As an example, one of the key questions that physiologists face is how will forests respond to rapidly changing weather patterns and atmospheric carbon dioxide levels? It is now clear from a wide body of literature that the capacity of forests to grow and adsorb extra C in response to increasing atmospheric carbon dioxide concentrations will likely be regulated by ecosystem nutrient cycling, particularly of N (e.g., Johnson 2006). While there are thousands of studies that have quantified changes in the C physiology of trees in response to elevated atmospheric CO_2 (including many reviews), there are only scores that have dealt with forest ecosystem nutrient cycling and only a handful that have addressed N storage and remobilization within the trees themselves, all of these in young seedlings (e.g., Temperton et al. 2003, Dyckmans and Flessa 2005, Vizoso et al. 2008).

Given that N cycling is likely to ultimately regulate ecosystem scale responses, this is potentially a major gap in our understanding of how trees respond to elevated CO_2 .

Acknowledgments

P.M. thanks the Scottish Government's Rural and Environmental Research and Analysis Directorate and Landcare Research for funding. G-A.G. thanks the Natural Environment Research Council for funding. We also thank David Whitehead for his helpful comments on the manuscript.

References

- Adams, H.H., M. Guardiola-Claramonte, G.A. Barron-Gafford, J.C. Villegas, D.D. Breshears, C.B. Zou, P.A. Troch and T.E. Huxman. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proc. Natl Acad. Sci. USA* 106:7063–7066.
- Aerts, R. 1990. Nutrient use efficiency in evergreen and deciduous species from heathlands. *Oecologia* 84:391–397.
- Aerts, R. 1995. The advantages of being evergreen. *Trends Ecol. Evol.* 10:402–407.
- Aerts, R. and F.S. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv. Ecol. Res.* 30:1–67.
- Akselsson, C., O. Westling, M. Alvetag, G. Thelin, A.-M. Fransson and S. Hellsten. 2008. The influence of N load and harvest intensity on the risk of P limitation in Swedish forest soils. *Sci. Total Environ.* 404:284–289.
- Ameglio, T., M. Decourteix, G. Alves, V. Valentin, S. Sakr, J.L. Julienne, G. Pétel, A. Guillot and A. Lacoite. 2004. Temperature effects on xylem sap osmolarity in walnut trees: evidence for a vitalistic model of winter embolism repair. *Tree Physiol.* 24:785–793.
- Berendse, F. and R. Aerts. 1987. Nitrogen-use-efficiency: a biologically meaningful definition? *Funct. Ecol.* 1:293–296.
- Birk, E.M. and P.M. Vitousek. 1986. Nitrogen availability and nitrogen use efficiency in loblolly-pine stands. *Ecology* 67:69–79.
- Birkhold, K.T. and R.L. Darnell. 1993. Contribution of storage and currently assimilated nitrogen to vegetative and reproductive growth of rabbiteye blueberry. *J. Am. Soc. Hortic. Sci.* 118: 101–108.
- Bhupinderpal-Singh, A. Nordgren, M.O. Lofvenius, M.N. Höbger, P.E. Mellander and P. Höbger. 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell Environ.* 26:1287–1296.
- Bréda, N., R. Huc, A. Granier and E. Dreyer. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann. For. Sci.* 63:625–644.
- Bryant, J.P., F.S. Chapin and D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368.
- Camm, E. 1993. Photosynthetic responses in developing and year-old Douglas-fir needles during new shoot development. *Trees* 8:61–66.
- Carswell, F.E., P. Millard, G.N.D. Rogers and D. Whitehead. 2003. Influence of nitrogen and phosphorous supply on foliage growth and internal recycling of nitrogen in conifer seedlings (*Prunopitys ferruginea*). *Funct. Plant Biol.* 30:49–55.

- Chapin, F.S., E.-D. Schulze and H.A. Mooney. 1990. The ecology and economics of storage in plants. *Annu. Rev. Ecol. Syst.* 21:423–447.
- Cheng, L.L. and L.H. Fuchigami. 2002. Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiol.* 22:1297–1303.
- van Cleve, B. and K. Apel. 1993. Induction by nitrogen and low temperature of storage-protein synthesis in poplar trees exposed to long days. *Planta* 189:157–160.
- Coleman, G.D., T.H.H. Chen, S.G. Ernst and L. Fuchigami. 1991. Photoperiod control of poplar bark storage protein accumulation. *Plant Physiol.* 96:686–692.
- Cooke, J.E.K. and M. Weih. 2005. Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. *New Phytol.* 167:19–30.
- Côté, B. and C. Camiré. 1987. Tree growth and nutrient cycling in dense plantations of hybrid poplar and black alder. *Can. J. For. Res.* 17:516–523.
- Crête, M. and G.J. Doucet. 1998. Persistent suppression in dwarf birth after release from heavy summer browsing by caribou. *Arctic Alpine Res.* 30:126–132.
- Deng, X., S.A. Weinbaum, T.M. DeJong and T.T. Muraoka. 1989. Utilization of nitrogen from storage and current-year uptake in walnut spurs during the spring flush of growth. *Physiol. Plant.* 75:492–498.
- Dungan, R.J., M.H. Turnbull and D. Kelly. 2007. The carbon cost for host trees of a phloem-feeding herbivore. *J. Ecol.* 95:603–613.
- Drake, B.G., M.A. Gonzalez-Meler and S.P. Long. 1997. More efficient plants: a consequence of rising atmospheric CO₂? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:609–639.
- Dyckmans, J. and H. Flessa. 2001. Influence of tree internal N status on uptake and translocation of C and N in beech: a dual ¹³C and ¹⁵N labelling approach. *Tree Physiol.* 21:395–401.
- Dyckmans, J. and H. Flessa. 2005. Partitioning of remobilised N in young beech (*Fagus sylvatica* L.) is not affected by elevated [CO₂]. *Ann. For. Sci.* 62:285–288.
- Frak, E.W., P. Millard, X. Le Roux, S. Guillaumie and R. Wendler. 2002. Coupling sap flow velocity and amino acid concentrations as an alternative method to ¹⁵N labeling for quantifying nitrogen remobilization by walnut trees. *Plant Physiol.* 130:1043–1053.
- Gansert, D. and W. Sprick. 1998. Storage and mobilization of non-structural carbohydrates and biomass development of beech seedlings (*Fagus sylvatica* L.) under different light regimes. *Trees* 12:247–257.
- Gill, R.M.A. 2006. The influence of large herbivores on tree recruitment and -forest dynamics. In *Large Herbivore Ecology and Ecosystem Dynamics*. Eds. K. Danell, R. Bergström, P. Duncan and J. Pastor. Cambridge University Press, Cambridge, pp 170–202.
- Glavac, V. and H. Jochheim. 1993. A contribution to understanding the internal nitrogen budget of beech (*Fagus sylvatica* L.). *Trees* 7:237–241.
- Grassi, G., P. Millard, R. Wendler, G. Minotta and M. Tagliavini. 2002. Measurement of xylem sap amino acid concentrations in conjunction with whole tree transpiration estimates spring N remobilisation by cherry (*Prunus avium* L.) trees. *Plant Cell Environ.* 25:1689–1699.
- Grelet, G.-A., I.J. Alexander, M.F. Proe and P. Millard. 2001. Leaf habit influences nitrogen remobilization in *Vaccinium* species. *J. Exp. Bot.* 52:993–1002.
- Guak, S., D. Neilsen, P. Millard and G.H. Neilsen. 2003. Determining the role of N remobilization for growth of apple (*Malus domestica* Borkh.) trees by measuring xylem-sap flux. *J. Exp. Bot.* 54: 2121–2131.
- Helmisaari, H.-S. 1992. Nutrient translocation in three *Pinus sylvestris* stands. *For. Ecol. Manage.* 51:347–367.
- Hoch, G. 2005. Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. *Plant Cell Environ.* 28:651–659.
- Hoch, G. and C. Körner. 2003. The carbon charging of pines at the climatic treeline; a global comparison. *Oecologia* 135:10–21.
- Hoch, G., A. Richter and C. Körner. 2003. Non-structural carbon compounds in temperate forest trees. *Plant Cell Environ.* 26: 1067–1081.
- Högberg, P., M.O. Löfvenius Bhupinder-Singh and A. Nordgren. 2009. Partitioning of soil respiration into autotrophic and heterotrophic components by means of tree-girdling in old boreal spruce forest. *For. Ecol. Manage.* 257:1764–1767.
- Honkanen, T., E. Haukioja and V. Kitunen. 1999. Responses of *Pinus sylvestris* branches to simulated herbivory are modified by tree source/sink dynamics and by external resources. *Funct. Ecol.* 13:126–140.
- Hoogesteger, J. and P.S. Karlsson. 1992. Effects of defoliation on radial stem growth and photosynthesis in the mountain birch (*Betula pubescens* ssp. *tortuosa*). *Funct. Ecol.* 6:317–323.
- Johnson, D.W. 2006. Progressive N limitation in forests: review and implications for long-term responses to elevated CO₂. *Ecology* 87:64–75.
- Kagawa, A., A. Sugimoto and T.C. Maximov. 2006a. Seasonal course of translocation, storage and remobilization of ¹³C pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings. *New Phytol.* 171:793–804.
- Kagawa, A., A. Sugimoto and T.C. Maximov. 2006b. ¹³CO₂ pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant Cell Environ.* 29:1571–1584.
- Körner, C. 2003. Carbon limitation in trees. *J. Ecol.* 91:4–17.
- Körner, C. 2006. Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytol.* 172:393–411.
- Lal, C.B., C. Annapurna, A.S. Raghubanshi and J.S. Singh. 2001. Foliar demand and resource economy of nutrients in dry tropical forest species. *J. Veg. Sci.* 12:5–14.
- Langheinrich, U. and R. Tischner. 1991. Vegetative storage proteins in poplar—induction and characterisation of a 32-kilodalton and a 36-kilodalton polypeptide. *Plant Physiol.* 97:1017–1025.
- Le Goff, N. and J.-M. Ottorini. 2001. Root biomass and biomass increment in a beech (*Fagus sylvatica* L.) stand in northeast France. *Ann. For. Sci.* 58:1–13.
- Lehtilä, K., E. Haukioja, P. Kaitaniemi and P.A. Laine. 2000. Allocation of resources within mountain birch canopy after simulated winter browsing. *Oikos* 90:160–170.
- Li, M.H., G. Hoch and C. Körner. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees* 16:331–337.
- Long, S.P., E.A. Ainsworth, A. Rogers and D.R. Ort. 2004. Rising atmospheric carbon dioxide: plants face the future. *Annu. Rev. Plant Biol.* 55:591–628.
- Mailk, V. and V.R. Timmer. 1997. Biomass partitioning and nitrogen retranslocation in black spruce seedlings on competitive mixed-wood sites: a bioassay study. *Can. J. For. Res.* 28:206–214.
- Malaguti, D., P. Millard, R. Wendler, A. Hepburn and M. Tagliavini. 2001. Translocation of amino acids in the xylem of apple (*Malus domestica* Borkh.) trees in spring as a consequence of both N remobilization and root uptake. *J. Exp. Bot.* 52:1665–1671.
- Manter, D.K., K.L. Kavanagh and C.L. Rose. 2005. Growth response of Douglas-fir seedlings to nitrogen fertilization: importance of Rubisco activation state and respiration rates. *Tree Physiol.* 25:1015–1021.

- Marty, C., T. Lamaze and A. Pornon. 2009. Endogenous sink–source interactions and soil nitrogen regulate leaf life-span in an evergreen shrub. *New Phytol.* 183:1114–1123.
- Maurel, K., G.B. Leite, M. Bonhomme, A. Guilliot, R. Rageau, G. Pétel and S. Sakr. 2004. Trophic control of bud break in peach (*Prunus persica*) trees: a possible role of hexoses. *Tree Physiol.* 24:579–588.
- McDowell, N., W.T. Pockman, C.D. Allen et al. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178:719–739.
- Millard, P. 1988. The accumulation and storage of nitrogen by herbaceous plants. *Plant Cell Environ.* 11:1–8.
- Millard, P. 1993. A review of internal cycling of nitrogen within trees in relation to soil fertility. In *Optimisation of Plant Nutrition*. Eds. M.A.C. Fragoso, M.L. Van Beusichem and A. Houwers. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 623–628.
- Millard, P. 1994. Measurement of the remobilization of nitrogen for spring leaf growth of trees under field conditions. *Tree Physiol.* 14:1049–1054.
- Millard, P. and G.H. Nielsen. 1989. The influence of nitrogen supply on the uptake and remobilization of stored N for the seasonal growth of apple trees. *Ann. Bot.* 63:301–309.
- Millard, P. and M.F. Proe. 1991. Leaf demography and the seasonal internal cycling of nitrogen in sycamore (*Acer pseudoplatanus* L.) seedlings in relation to nitrogen supply. *New Phytol.* 117:587–596.
- Millard, P. and M.F. Proe. 1992. Storage and internal cycling of nitrogen in relation to seasonal growth of Sitka spruce. *Tree Physiol.* 10:33–43.
- Millard, P. and M.F. Proe. 1993. Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bog.) Carr. as influenced by nitrogen supply. *New Phytol.* 125:113–119.
- Millard, P. and C.M. Thomson. 1989. The effect of autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple trees. *J. Exp. Bot.* 40:1285–1289.
- Millard, P., R. Wendler, A. Hepburn and A. Smith. 1998. Variations in the amino acid composition of xylem sap of *Betula pendula* Roth. trees due to remobilization of stored N in the spring. *Plant Cell Environ.* 21:715–722.
- Millard, P., A. Hester, R. Wendler and G. Baillie. 2001. Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Funct. Ecol.* 15:535–543.
- Millard, P., R. Wendler, G. Grassi, G.-A. Grelet and M. Tagliavini. 2006. Translocation of nitrogen in the xylem of field-grown cherry and poplar trees during remobilisation. *Tree Physiol.* 26:527–536.
- Millard, P., M. Sommerkorn and G.-A. Grelet. 2007. Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. *New Phytol.* 175:11–28.
- Miller, H.G. 1986. Carbon × nitrogen interactions—the limitations to productivity. *Tree Physiol.* 2:373–385.
- Miller, H.G. and J.D. Miller. 1987. Nutritional requirements of Sitka spruce. *Proc. R. Soc. Edinb.* 85B:83–96.
- Millett, J., P. Millard, A.J. Hester and A.J.S. McDonald. 2005. Do competition and herbivory alter the internal nitrogen dynamics of birch saplings? *New Phytol.* 168:413–422.
- Nambiar, E.K.S. 1987. Do nutrients retranslocate from fine roots? *Can. J. For. Res.* 17:913–918.
- Nambiar, E.K.S. and D.N. Fife. 1987. Growth and nutrient retranslocation in needles of radiata pine in relation to nitrogen supply. *Ann. Bot.* 60:147–156.
- Näsholm, T. 1994. Removal of nitrogen during needle senescence in Scots pine (*Pinus sylvestris* L.). *Oecologia* 99:290–296.
- Näsholm, T., K. Kielland and U. Ganeteg. 2008. Uptake of organic nitrogen by plants. *New Phytol.* 182:31–48.
- Neilsen, D., P. Millard, G.H. Nielsen and E.J. Hogue. 1997. Sources of N used for leaf growth in a high density apple (*Malus domestica*) orchard irrigated with ammonium nitrate solution. *Tree Physiol.* 17:733–739.
- Palacio, S., A.J. Hester, M. Maestro and P. Millard. 2008. Browsed *Betula pubescens* trees are not carbon-limited. *Funct. Ecol.* 22:808–815.
- Pfautsch, S., A. Gessler, M.A. Adams and H. Rennenberg. 2009. Using amino-nitrogen pools and fluxes to identify contributions of understory *Acacia* spp. to overstory *Eucalyptus regnans* and stand nitrogen uptake in temperate Australia. *New Phytol.* 183:1097–1113.
- Polcarpo, M., L. Di Marco, T. Caruso, P. Gioacchini and M. Tagliavini. 2002. Dynamics of nitrogen uptake and partitioning in early and late fruit ripening peach (*Prunus persica*) tree genotypes under a Mediterranean climate. *Plant Soil* 239:207–214.
- Pornon, A. and T. Lamaze. 2007. Nitrogen resorption and photosynthetic activity over leaf life span in an evergreen shrub, *Rhododendron ferrugineum*, in a subalpine environment. *New Phytol.* 175:301–310.
- Proe, M.F., A.J. Midwood and J. Craig. 2000. Use of stable isotopes to quantify nitrogen, potassium and magnesium dynamics in young Scots pine (*Pinus sylvestris*). *New Phytol.* 146:461–469.
- Quartieri, M., P. Millard and M. Tagliavini. 2002. Storage and remobilisation of nitrogen by pear (*Pyrus communis* L.) trees as affected by timing of N supply. *Eur. J. Agron.* 17:105–110.
- Read, D.J. and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? *New Phytol.* 157:475–492.
- Rennenberg, H., M. Dannenmann, A. Gessler, J. Kreuzwieser, J. Simon and H. Papen. 2009. Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. *Plant Biol.* 11:4–23.
- Rogers, A. and D.S. Ellsworth. 2002. Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO₂ (FACE). *Plant Cell Environ.* 25:851–858.
- Salifu, K.F. and V.R. Timmer. 2003. Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Sci. Soc. Am. J.* 67:309–317.
- Sanz-Pérez, V.P., P. Castro-Díez and R. Joffre. 2009. Seasonal carbon storage and growth in Mediterranean tree seedlings under different water conditions. *Tree Physiol.* 29:1105–1116.
- Sauter, J.J. and B. van Cleve. 1994. Storage, mobilization and interrelations of starch, sugars, protein and fat in the ray storage tissue of poplar trees. *Trees* 8:297–304.
- Schädel, C., A. Blöchl, A. Richter and G. Hoch. 2009. Short-term dynamics of non-structural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. *Tree Physiol.* 29:901–911.
- Schmidt, S. and G.R. Stewart. 1998. Transport, storage and mobilization of nitrogen by trees and shrubs in the wet/dry tropics of northern Australia. *Tree Physiol.* 18:403–410.
- Schneider, S., J. Kreuzwieser, R. Schums, J.J. Sauter and H. Rennenberg. 1994. Thiol and amino acid composition of the xylem sap of poplar trees. *Can. J. Bot.* 72:347–351.
- Schutz, A.E., W.J. Bond and M.D. Cramer. 2009. Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. *Oecologia* 160:235–246.
- Silla, F. and A. Escudero. 2003. Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species. *Oecologia* 136:28–36.

- Silla, F. and A. Escudero. 2004. Nitrogen-use efficiency: trade-offs between N productivity and mean residence time at organ, plant and population levels. *Funct. Ecol.* 18:511–521.
- Silla, F. and A. Escudero. 2006. Coupling N cycling and N productivity in relation to seasonal stress in *Quercus pyrenaica* Willd. saplings. *Plant Soil* 282:301–311.
- Spann, T.M., R.H. Beede and T.M. DeJong. 2008. Seasonal carbohydrate storage and mobilization in bearing and non-bearing pistachio (*Pistacia vera*) trees. *Tree Physiol.* 28:207–213.
- Stephens, D.W., P. Millard, M.H. Turnbull and D. Whitehead. 2001. The influence of nitrogen supply on growth and internal recycling of nitrogen in young *Nothofagus fusca* trees. *Aust. J. Plant Physiol.* 28:249–255.
- Tagliavini, M., M. Quartieri and P. Millard. 1997. Remobilised nitrogen and root uptake of nitrate for spring leaf growth, flowers and developing fruits of pear (*Pyrus communis* L.) trees. *Plant Soil* 195:137–142.
- Talbot, J.M., S.D. Allison and K.K. Treseder. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Funct. Ecol.* 22:955–963.
- Temperton, V., P. Millard and P.G. Jarvis. 2003. Does elevated atmospheric carbon dioxide affect internal nitrogen allocation in the temperate trees *Alnus glutinosa* and *Pinus sylvestris*? *Glob. Chang. Biol.* 9:286–294.
- Thomas, M.M., P. Millard, M.S. Watt, M.H. Turnbull, D. Peltzer and D. Whitehead. 2008. The impact of defoliation on nitrogen translocation patterns in the woody invasive plant, *Buddleia davidii*. *Funct. Plant Biol.* 35:462–469.
- Tian, W.M., J.L. Wu, B.Z. Hao and Z.H. Hu. 2003. Vegetative storage proteins in the tropical tree *Swietenia macrophylla*: seasonal fluctuations in relation to a fundamental role in the regulation of tree growth. *Can. J. Bot.* 81:492–500.
- Tissue, D.T., K.L. Griffin, M.H. Turnbull and D. Whitehead. 2001. Canopy position and needle age affect photosynthetic response in field-grown *Pinus radiata* after five years of exposure to elevated carbon dioxide partial pressure. *Tree Physiol.* 21: 915–923.
- Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. *Am. Nat.* 119:553–572.
- Vizoso, S., D. Gerant, J.M. Guehl, R. Joffre, M. Chalot, P. Gross and P. Maillard. 2008. Do elevation of CO₂ concentration and nitrogen fertilization alter storage and remobilization of carbon and nitrogen in pedunculate oak saplings? *Tree Physiol.* 28: 1729–1739.
- Wardle, D.A., L.R. Walker and R.D. Bardgett. 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science* 305:509–513.
- Weinbaum, S. and C. Van Kessel. 1998. Quantitative estimates of uptake and internal cycling of ¹⁴N-labeled fertilizer in mature walnut trees. *Tree Physiol.* 18:795–801.
- Wendler, R. and P. Millard. 1996. Impacts of water and nitrogen supplies on the physiology, leaf demography and nitrogen dynamics of *Betula pendula*. *Tree Physiol.* 16:153–159.
- Wendler, R., P.O. Carvalho, J.S. Pereira and P. Millard. 1995. Role of nitrogen remobilization from old leaves for new leaf growth of *Eucalyptus globulus* seedlings. *Tree Physiol.* 15:679–683.
- Würth, M.K.R., S. Peláez-Riedl, S.J. Wright and C. Körner. 2005. Non-structural carbohydrate pools in a tropical forest. *Oecologia* 143:11–24.
- Zhu, B. and G.D. Coleman. 2001. Phytochrome-mediated photoperiod perception, shoot growth, glutamine, calcium, and protein phosphorylation influence the activity of the poplar bark storage protein gene promoter (bspA). *Plant Physiol.* 126: 342–351.